

In-Line Solid-Phase Extraction for Sensitivity Enhancement in Capillary Electrophoresis

by

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of

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DECLARATION

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25 March 2009

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LIST OF ABBREVIATIONS

BET	Bremmer-Emmet-Teller
BGE	Background electrolyte
CE	Capillary Electrophoresis
CEC	Capillary electrochromatography
CZE	Capillary zone electrophoresis
EOF	Electroosmotic flow
FTIR	Fourier transform infra red
i.d.	Internal diameter
IE	Ion exchange
Lcc	Localised column capacity
MA	Methacrylic acid
o.d.	Outer diameter
ODS	Octadecyl silica
OT	Open tubular
RP	Reversed phase
SEM	Scanning electron microscopy
SPE	Solid-phase extraction
UV	Ultra violet
WCX	Weak cation exchange
γ -MAPS	3-(methacryloyloxypropyl)trimethoxy silane

LIST OF PUBLICATIONS

Type of Publication	Number	Reference
Papers in refereed journals	5	1-5
Refereed posters at local and international meetings	3	6,8,10
Contributions to talks at international and local meetings	2	7,9

1. Thabano J.R.E., Breadmore M.C., Johns C., Hutchinson J.P., Haddad P.R., **Capillary Electrophoresis of Neurotransmitters using In-Line Solid-Phase Extraction and Preconcentration using a Methacrylate-Based Weak Cation-Exchange Monolithic Stationary Phase and a pH Step Gradient**, *J.Chromatogr. A*, 1175, 2007, 117-126 (Chapter 4).
2. Thabano J.R.E., Breadmore M.C., Johns C., Hutchinson J.P., Haddad P.R., **Selective Extraction and Elution of Weak Bases by In-Line Solid-Phase Extraction Capillary Electrophoresis using a pH Step Gradient and a Weak Cation-Exchange Monolith**, *Analyst* 133, 2008, 1380-1387 (Chapter 5).
3. Thabano J.R.E., Breadmore M.C., Johns C., Hutchinson J.P., Haddad P.R., **Silica Nanoparticles Templated Methacrylic acid Polymer Monoliths for In-Line Solid-Phase Extraction Capillary Electrophoresis of Basic Analytes**, *J.Chromatogr. A* 2008, accepted (Chapter 6).
4. Breadmore M.C., Thabano J.R.E., Dawod M., Kazarian A.A., Quirino J.P., Guijt R.M., **Recent Advances in Preconcentration on Capillary Electrophoresis and Microchips**, *Electrophoresis* 30 (2008), 1-19 (Chapter 1).
5. Thabano J.R.E., Breadmore M.C., Quirino J.P., **Mechanisms of the pH Junction using Low pH Buffers and Weak Bases as Analytes: Experimental and Theoretical Considerations**, *Anal. Chem*, under preparation (related work).
6. Thabano J.E.R., Breadmore M.C. and Haddad P.R., **Ion-Exchange Preconcentration on a Weak Cation Exchange Monolithic Column using pH Step Gradients**. *21st International Symposium on Microscale BioSeparations 2007 (MSB 2007)*, Vancouver, Canada, 14-18 January 2007 (Poster).
7. Thabano J.E.R., Breadmore M.C., Johns C.A., Hutchinson J.P. and Haddad P.R., **Preconcentration and Solid-Phase Extraction on a Weak Cationic Exchange Monolithic Column with Elution using a pH Step Gradient**, *14th Annual*

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9. Thabano J.R.E., Breadmore M.C., Johns C., Hutchinson J.P., Haddad P.R., **Development and Characterisation of Polymeric Monoliths for In-Line Solid-Phase Extraction Capillary Electrophoresis**, *Inaugural UTAS Postgraduate Research Conference: Sharing Excellence in Research*, 21 September 2007 (Lecture).
10. Thabano J.R.E., Breadmore M.C., Johns C., Hutchinson J.P., Haddad P.R., **A Novel In-Line Preconcentration Method on a Weak Cation Exchange Monolith**, *School of Chemistry Research Evening*, 26 October 2006 (Poster).

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ABSTRACT

This work presents a systematic study into the use of weak acid monolithic columns and moving pH boundaries for in-capillary solid-phase extraction – capillary electrophoresis.

A new methacrylic porous polymer monolith was prepared via photoinitiated free radical polymerisation both in bulk and inside TeflonTM coated 75 μm i.d. x 365 μm o.d. capillaries. Bulk monoliths were used for characterisation, and it was found that the pore size properties of the monolith could be controlled from 1.4 μm to 3 μm by variation of the monomer content. Comparison with thermally polymerised monoliths prepared using the same monomers, cross linker and porogens, had a much lower surface area, 6 m^2/g compared to 24 m^2/g for the photoinitiated monoliths. The physical and chemical characteristics of these porous polymer monoliths were measured by charge-coupled device camera, scanning electron microscopy, infra-red spectroscopy, BET surface area and titration experiments. In the case of scanning electron microscopy and charge-coupled device camera the successful anchoring and morphology of the polymer monolith was confirmed. The scanning electron microscopy analysis of the porous polymer monolith also showed that the change in morphology is very small and cannot be detected by analogy of the images. The BET surface area of these porous polymer monoliths ranged 12-24 m^2/g . The carboxylic acid functionality in both polymer solutions and final polymer were confirmed by IR at 3500 and 1720 cm^{-1} for $\nu_{\text{C=O}}$ and ν_{OH} respectively. The carboxylic acid group was further confirmed by titration of the bulk monolith and the pKa typical of a carboxylic group on solid surface at 4.3.

This methacrylic acid monolith was fabricated inside capillaries and used for in-line solid-phase extraction-capillary electrophoresis. The advantage of having the solid phase extraction within the separation device was exploited to enable in-capillary preconcentration of neurotransmitters via ion-exchange interactions. A new elution method, namely the use of an electroosmotic flow mobilised pH step gradient, was introduced to protonate the monolith and was shown to efficiently elute the analytes from the preconcentration monolith. Due to the discontinuous nature of the electrolyte system, analytes were simultaneously eluted and focused as the electrophoretically

mobilised pH step boundary traversed past the monolith, after which the analytes were separated according to their electrophoretic mobilities in the open section of the capillary. A fundamental study of the generation and implementation of the pH step was undertaken using dopamine and epinephrine as test analytes. Optimisation of the pH and concentration of binding electrolyte established best adsorption using 6 mM phosphate at pH 7, containing 12 mM sodium ion, which is above the pKa of the monolith but below the pKa of the analytes. Optimisation of the elution electrolyte yielded the best results when 12 mM phosphate pH 3, containing 12 mM sodium ion was used. Under these optimum conditions the sensitivity of simple neurotransmitters could be improved by over 500 times that of a normal hydrodynamic injection in CE. The analytical potential of the developed solid-phase extraction-capillary electrophoresis method was demonstrated with the detection of dopamine in a 3 times diluted urine sample from a healthy volunteer.

The selectivity of the solid-phase extraction device was also tested with analytes having differing pKa values. A series of weak bases, namely imidazole, benzylamine, lutidine and 3-phenylpropanamine, with pKa values ranging from 6 to 10 were selected to examine the ability of the solid-phase extraction monolith to selectively elute and concentrate these weak bases. This was demonstrated by performing pH selective extraction, in which a pH was selected at which only certain analytes were extracted, and pH selective removal, in which unwanted analytes were removed after extraction and prior to the separation. In addition, four different electrolytes containing phosphate, formate, acetate or citrate were tested. Acetate was found to give the narrowest peak width when measured at base, using benzylamine as analyte. After optimisation of the conditions necessary for maximum focusing 44 mM acetate pH 6 and 3 mM acetate pH 3 were found as the optimum providing a peak width of 1.5 s. Using the optimum conditions a 0.25 µg/ml test mixture of imidazole (pKa 6.99), lutidine (pKa 6.63) and 3-phenylpropanamine (pKa 10.28) was introduced at pH 6. pH selective removal was achieved by hydrodynamically introducing an acetate electrolyte at pH 9 containing 60% acetonitrile successfully removing both imidazole and lutidine, prior to applying the electroosmotic flow mobilised pH step and separation which successfully confirmed the presence of only phenylpropanamine. The versatility of this approach was

investigated further by performing pH selective extraction where the test mixture this time was loaded in the pH 9 electrolyte containing similar amount of organic modifier, successfully extracting only phenylpropanamine in the presence of imidazole and lutidine. The overall recoveries obtained using both this approaches were ranging 60% to 110%, indicating the applicability of this approaches for analytical purposes.

Finally, one of the main limitations of methacrylate-based monoliths, namely their low surface area, has been examined by templating the monoliths during polymerisation with 80 nm silica nanoparticles, which are subsequently removed by dissolving with sodium hydroxide. On varying the amount of silica nanoparticles in the polymer solution and etching time the overall capacity of the polymer monolith could be increased by 16 times when compared to the original monolith. When used for solid-phase extraction, this enabled higher loading times which translated to a reduction in the detection limits by up to 1900 times compared to a normal injection in CE. The potential of this increased capacity was demonstrated by direct loading of raw samples from biological (urine), environmental and food, without any pretreatment.